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SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM 1/

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DETERMINATION OF THEBAINE IN PAPAVER BRACTEATUM 2/

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1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

3/ This paper was submitted by Dr. J.A. Snyder, Market Research Manager, Pharmaceuticals Division, Biochemical Department, E.I. du Pont de Nemours & Co. Inc., Wilmington, Delaware 19898, U.S.A.

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Preparation of plant material prior to assay

Grind the sample with a suitable mill[4] to pass a 40 mesh screen. Dry the ground plant material to constant weight at 60°C in air. Store the dried, ground sample in a desiccator under vacuum.

Preparation of sample residues for chromatography

Transfer a portion of the ground, dried plant material, accurately weighed (refer to Table I), into a stoppered flask. Add 30.0 ml 3.0% (v/v) acetic acid and shake for 20 minutes. Filter through a medium porosity fritted glass funnel with the aid of vacuum, collecting the filtrate in a 125-ml separator. Return the plant material to the flask and extract as before with 20.0 ml 3.0% (v/v) acetic acid. After the second extraction, rinse the funnel with 5 ml 3.0% (v/v) acetic acid, collecting the washings in the separator.

Adjust the pH of the combined extracts and washings to 8.7-9.3 (check with short-range pH paper), using ammonia:ammonium chloride buffer[5]. Typically, 15-20 ml are required to raise the pH to the desired range. Extract with two 25-ml portions of benzene. Shake the separator gently since samples tend to form emulsions during the extraction. If the emulsion does not break on standing, allow the contents of the separator to drain slowly into a second separator. Now the emulsion should have broken sufficiently to allow separation of the benzene phase. Pass the benzene phase through a pledget of benzene-wetted glass wool that has been previously washed with benzene and collect these extracts in a 100-ml round-bottom flask. Rinse the glass wool with a few ml of benzene, adding the rinsings to the extracts. Evaporate the combined extracts and rinsings to dryness on a suitable rotary evaporator with the aid of vacuum[6]. The residue thus obtained is the sample residue.

4/ e.g. Wiley Laboratory Mill, Model PRI. Available from A.H. Thomas Co., Philadelphia, Pa.

5/ pH = 9.5, 2.5M NH_3 and 2.5M NH_4Cl (the buffer is prepared by dissolving 33.5 g NH_4Cl in about 150 ml water, adding 42 ml concentrated ammonia and diluting to 250 ml with water).

6/ e.g. Buchi Rotavapor R with heating bath at 35-40°C. Available from Brinkmen Instruments, Inc., Westbury, New York.

Table I

	For thebaine content less than 1%, use	For thebaine content greater than 1%, use
Dried ground plant material	500 mg	200 mg
Aliquots of thebaine stock standard	3.00, 6.00 & 10.00 ml	6.00, 10.00 & 15.00 ml
Internal standard	2.00 ml	4.00 ml

Preparation of standard residues for chromatography

Transfer 50 mg of thebaine reference standard, accurately weighed, into a 100-ml volumetric flask. Dissolve and bring to volume with 3.0% (v/v) acetic acid. Prepare three standards of varying concentration by pipetting aliquots of this stock solution (refer to Table I) into separate 125-ml separators. Add sufficient 3.0% (v/v) acetic acid to bring the total aqueous volume in each separator to 55 ml.

Proceed as directed in preparation of sample residues for chromatography, beginning with the phrase "Adjust the pH ...". The residues thus obtained are the standard residues.

Internal standard preparation

The internal standard is an n-butanol solution of cholesterol acetate at a concentration of 1.0 mg per ml.

Gas chromatography of standards and samples

Dissolve each residue in an identical volume of internal standard (refer to Table I). Inject the most concentrated standard on a suitable gas chromatograph[7/], equipped with a flame ionization detector at the conditions suggested below, until successive injections give thebaine:internal standard peak area ratios that agree to better than two percent.

7/ e.g. Hewlett-Packard, Model 402. Available from Hewlett-Packard, Paramus, New Jersey.

Column:	4' x 3.5 mm, I.D. glass column with 3.8% OV-17 Gas Chrom Q, 100-120 mesh
Oven:	250°C
Flash Heater:	280°C
Detector:	290°C
He (carrier) Flow Rate:	about 75 ml/minute
H_2 Flow Rate:	about 30 ml/minute
Air Flow Rate:	about 350 ml/minute
Chart Speed:	1/4 inch/minute
Approximate Retention:	4 minutes: thebaine 11 minutes: cholesterol acetate.

Typically it is found that a 2 µl injection will give the desired response and that two to four injections are necessary to achieve the desired precision. It is sometimes found that data from the first few injections should be omitted because the column response is not stabilized. Inject each remaining standard twice and then each sample once in turn. Chromatograms of some plant material show extra peaks after the internal standard. Therefore, injections should be made at least 20 minutes apart for the first injection of all samples. Each sample is to be injected twice, or until a 2 percent coefficient of variation is obtained for the peak area ratio of that sample. After every eight sample injections (or more frequently if necessary), alternately inject a standard. After all samples, make a final injection of each standard.

Calculation of thebaine content

Compute a mean thebaine:internal standard peak area ratio for each standard and sample. Make a linear plot from the standard data of the amount of thebaine (mg/round-bottom flask) versus mean peak area ratio. Interpolate the weight (mg) of thebaine in each plant sample from the mean peak area ratio found for the sample, and compute the thebaine content by:

$$\text{Percent thebaine in plant sample} = \frac{\text{Thebaine found (mg)}}{\text{Anhydrous sample Wt. (mg)}} \times 100$$

Analytical data on P. bracteatum samples

Plant material	Number	% thebaine (anhydrous)	Capsule weight (g)
UNB-17 Reference sample (powder)	Run 1	3.40	-
	2	3.39	-
	3	3.43	-
	4	3.41	-
	Mean	3.41	-
Arya II capsules (Prof. Lalezari)	1	3.56	2.23
	2	2.60	1.27
	3	3.96	2.54
	4	--	1.31
	5	5.84	1.95
	6	--	1.88
	7	3.42	1.89
	8	3.52	1.33
	9 10	2.87 */	2.30
	11	5.40	3.03
	12	--	1.28
	13	--	1.30
	14	2.53	1.26
	15	--	1.10
	16	--	1.49
	17	4.07	1.42
	18	2.69	2.37
	19	--	1.94
	Mean	3.53	1.80

*/ Capsules 9 and 10 were combined after weighing and analyzed as a composite sample.